PATENT COOPERATION TREATY

09/869696

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

HARRISON GODDARD FOOTE
Tower House
Merrion Way
Leeds LS2 8PA
ROYAUME-UNI

13 JUN 2001 *056627

Date of mailing (day/month/year) 31 May 2001 (31.05.01)	
Applicant's or agent's file reference P15464WO1	IMPORTANT NOTIFICATION
International application No. PCT/GB99/04268	International filing date (day/month/year) 30 December 1999 (30.12.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
ML LABORATORIES
17 Hanover Square
London W1R 9AJ
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
ML LABORATORIES PLC
17 Hanover Square
London W1R 9AJ
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
I. Britel
Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004061991

TOTAL P.02

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

HARRISON GODDARD FOOTE
Tower House
Merrion Way
Leeds LS2 8PA
ROYAUME-UNI

Date of mailing (day/month/year)
10 July 2000 (10.07.00)

Applicant's or agent's file reference
P15464WO1

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/04268

International filing date (day/month/year)
30 December 1999 (30.12.99)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address

HARRISON GODDARD FOOTE
Belmont House
20 Wood Lane
Leeds LS6 2AE
United Kingdom

State of Nationality:
State of Residence:
Telephone No.: 44 113 225 8350
Facsimile No.: 44 113 230 4702
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

HARRISON GODDARD FOOTE
Tower House
Merrion Way
Leeds LS2 8PA
United Kingdom

State of Nationality:
State of Residence:
Telephone No.: 44 113 290 1400
Facsimile No.: 44 113 244 2829
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

I. Britel

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 11 August 2000 (11.08.00)	
International application No. PCT/GB99/04268	**Applicant's or agent's file reference** P15464WO1
International filing date (day/month/year) 30 December 1999 (30.12.99)	**Priority date** (day/month/year) 04 January 1999 (04.01.99)
Applicant DAVIES, Donald	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

19 July 2000 (19.07.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Olivia RANAIVOJAONA
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

GB9904268

[redacted]ENT COOPERATION TREATY

09/869696

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P15464W01		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 04268	30/12/1999	04/01/1999
Applicant		
ML LABORATORIES et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   P450 / ACETAMINOPHEN GDEPT FOR CANCER TREATMENT

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

[redacted] onal Application No
PC[redacted] 99/04268

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/53 A61K48/00 A61K31/165 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, MEDLINE, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 97 35994 A (GUENZBURG WALTER H ;SALLER ROBERT MICHAEL (DE); KARLE PETER (DE);) 2 October 1997 (1997-10-02) page 3, paragraph 2 - paragraph 3 page 5, paragraph 8 -page 6, paragraph 2 page 9, paragraph 3 page 10, paragraph 3 page 14, paragraph 1 -page 15, paragraph 3 ---	14
X	US 5 760 008 A (RUBIN DAVID) 2 June 1998 (1998-06-02) column 5, line 38 -column 6, line 33 column 7, line 21 - line 27 column 7, line 55 - line 58 column 16, line 56 -column 18, line 31 --- -/--	16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 2000	07/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sitch, W

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A .	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1992 AEBI S ET AL: "DIVERGENT EFFECTS OF INTRAVENOUS GSH AND CYSTEINE ON RENAL AND HEPATIC GSH" Database accession no. PREV199294112166 XP002140944 cited in the application abstract & AMERICAN JOURNAL OF PHYSIOLOGY, vol. 263, no. 2 PART 2, 1992, pages R348-R352, ISSN: 0002-9513 --- DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1991 PRINCIPE P ET AL: "CELLULAR THIOLS IN RAT LIVER CELL LINES POSSESSING DIFFERENT GROWTH CHARACTERISTICS" Database accession no. PREV199192050788 XP002140945 abstract & CELL BIOCHEMISTRY AND FUNCTION, vol. 9, no. 2, 1991, pages 125-134, ISSN: 0263-6484 --- DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1993 PERRY ROGER R ET AL: "Glutathione levels and variability in breast tumors and normal tissue." Database accession no. PREV199396089335 XP002140946 cited in the application abstract & CANCER (PHILADELPHIA), vol. 72, no. 3, 1993, pages 783-787, ISSN: 0008-543X --- -/--	
A		
A		

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A ,	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1993 PATTEN CHRIS J ET AL: "Cytochrome P450 enzymes involved in acetaminophen activation by rat and human liver microsomes and their kinetics." Database accession no. PREV199396085381 XP002140947 cited in the application abstract & CHEMICAL RESEARCH IN TOXICOLOGY, vol. 6, no. 4, 1993, pages 511-518, ISSN: 0893-228X -----	

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INTERNATIONAL SEARCH REPORT

[redacted]on patent family members

[redacted]tional Application No
P[redacted] 99/04268

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9735994 A	02-10-1997	AU 713382 B	02-12-1999
		AU 2382797 A	17-10-1997
		CA 2250173 A	02-10-1997
		CZ 9803050 A	13-01-1999
		EP 0892852 A	27-01-1999
		NO 984540 A	28-09-1998
		PL 329071 A	15-03-1999
		SK 132398 A	13-04-1999
US 5760008 A	02-06-1998	US 5639737 A	17-06-1997
		US 5476842 A	19-12-1995
		US 5340803 A	23-08-1994
		AU 692021 B	28-05-1998
		AU 4240796 A	10-07-1996
		CA 2208206 A	27-06-1996
		EP 0797453 A	01-10-1997
		WO 9619243 A	27-06-1996
		AT 178211 T	15-04-1999
		AU 665220 B	21-12-1995
		AU 3064192 A	07-06-1993
		CA 2122922 A	13-05-1993
		DE 69228829 D	06-05-1999
		DE 69228829 T	21-10-1999
		EP 0619704 A	19-10-1994
		ES 2132137 T	16-08-1999
		GR 3030259 T	31-08-1999
		MX 9206309 A	31-05-1994
		WO 9308688 A	13-05-1993

PATENT COOPERATION TREATY

09/869694

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P15464WO1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04268	30/12/1999	04/01/1999
International Patent Classification (IPC) or national classification and IPC A61K48/00		
Applicant ML LABORATORIES et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19/07/2000	21.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Sitch, W Telephone No. +31 70 340 3040

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/04268

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-28 as originally filed

Claims, No.:

1-20 as received on 22/01/2001 with letter of 16/01/2001

Drawings, No.:

1/8-8/8 as originally filed

Sequence listing part of the description, pages:

Figure 6, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☒ the claims, Nos.: 21
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 1-14, 17, 18 with respect to industrial applicability.

because:

   ☒ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14, 16-20
	No:	Claims	15
Inventive step (IS)	Yes:	Claims	1-14, 16-20
	No:	Claims	15
Industrial applicability (IA)	Yes:	Claims	15, 16, 19, 20
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 1-14, 17, 18 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO9735994

D2: US5760008

1. Novelty

Claim 15 relates to a vector capable of transfecting at least one tumour cell, wherein such vector includes at least one P450 gene or effective part thereof, whose expression is controlled by a promoter sequence or effective part thereof, and which shows substantially tumour cell specific expression.

D1 discloses a retroviral vector carrying a P450 gene (page 3, para. 2 - para. 3), for the activation of a prodrug for the treatment of tumour cells (page 5, para. 8 - page 6, para. 2, page 9, para. 3), and wherein the gene may be under control of target cell specific regulatory elements and promoters(page 10, para 3.). In a protocol for tumour treatment, vector is delivered, followed by a prodrug such as cyclophosphamide or ifosfamide (page 14, para. 1 - page 15, para. 3).

Accordingly, D1 discloses the combination of features of claim 14, the subject matter of which therefore lacks novelty under Art. 33(2), PCT.

2. Inventive Step.

Claim 1 is novel in light of the available prior art. D1, which may be considered as the closest prior art, discloses the treatment of tumours via delivery of a P450 gene and a prodrug such as cyclophosphamide. The essential difference between claim 1 and D1 is the delivery of acetaminophen as the prodrug.

The problem to be solved by the application is to provide an improved treatment of cancer, especially using GDEPT based techniques, and whereby improvements for example in the degree of selectivity of cancer cell destruction are achieved.

The cytotoxic effect of the metabolite N-acetyl benzoquinone on cancer cells is known from D2, such being produced by the action of a cytochrome P450 on acetaminophen (see for example D2, col. 5, line 38 - col. 6, line 33).

There would appear no motivation for the skilled person to attempt to ablate tumour cells in vivo by a method akin to that disclosed in D1, by substituting the prodrugs of D1 with the prodrug acetaminophen, with expectation of success in achieving selective destruction of tumour cells while limiting damage to other tissues such as liver. Inventive step (Art. 33(3) PCT) for claim 1 may thus be acknowledged. Since inventive step of claim 1 may be acknowledged, similarly inventive step for claims 2-14, 16-20 may also be acknowledged.

3. For the assessment of the present claims 1-20 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

Certain defects in the international application

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 and D2 are not mentioned in the description, nor are these documents identified therein.

2. Claim 14 has been assumed to be dependent on claims 1-13 and not 1-14 as given, and this International Preliminary Examination Report has been established under this assumption.

Re Item VIII

Certain observations on the international application

1. Claims 1-20 would appear not to be fully supported across their entire scope.

1.1 A treatment of cancer in general is made in claims 1-13, 17 and 18, with further specification thereof in claim 14. Support for such claims to the extent that the treatment is functional in tumour cells having a significantly lower glutathione content compared to that in liver, may be accepted. Based on that information given by the applicant, such a treatment would presumably not be applicable to tumours having a glutathione level similar to that found in liver cells however, and it is at present not clear that all tumours have the 'prerequisite' lower glutathione levels. In this way, support for the treatment of all tumours by the method of this application would appear to be lacking.

1.2 Claims 1-11, 14-20 relate to a P450 gene defined generically. In the context of the present application, the enzymes encoded by such a gene are involved in the oxidation of acetaminophen. Only certain P450 enzymes (for example those recited in claims 12 and 13) are involved in such a reaction however, and for this reason these claims wherein the gene encoding the enzyme is broadly defined would not appear fully supported across their entire scope.

Claims

1 A method for use in the treatment of cancer comprising:

i) administering to a mammal an effective amount of at least one vector capable of transfecting at least one tumour cell characterised in that said vector includes at least one P450 gene, or the effective part thereof, the expression of which is controlled by a promoter sequence, or the effective part thereof, which shows substantially tumour cell specific expression; and

ii) administering a therapeutically effective amount of at least acetaminophen, or a structurally related derivative thereof.

2. A method according to Claim 1 characterised in that said mammal is human.

3. A method according to Claims 1 or 2 characterised in that said vector is a eukaryotic expression vector.

4. A method according to any of Claims 1 – 3 characterised in that said vector is a viral based vector.

5. A method according to Claim 4 characterised in that said vector is a hybrid viral vector.

6. A method according to Claim 4 or 5 characterised in that said viral based vector is selected from at least one of the following: adenovirus; retrovirus;adeno associated virus; herpesvirus; lentivirus; or baculovirus.

7. A method according to any of Claims 1 – 6 characterised in that said tumour promoter is selected from at least one of : TRP-1; HER2; HER3; ERBB2; ERBB3; CEA; MUC1; or α-fetoprotein; Rous sarcoma virus long terminal repeat; cytomegalovirus promoter; murine leukaemia long terminal repeat; simian virus 40 early and late promoters; herpes simplex virus thymidine kinase promoter; prostate specific antigen promoter (PSA); zilin gene promoter; pancreatic amylase promoter; tyrosinase related peptide promoter; tumour rejection antigen precursor promoters.

8. A method according to Claim 7 characterised in that said promoter is a hybrid promoter of at least the effective parts of at least two tumour cell specific promoters.

9. A method according to any of Claims 1 – 8 characterised in that said P450 gene is of mammalian origin.

10. A method according to Claim 9 characterised in that said P450 gene is of human origin.

11. A method according to Claim 9 characterised in that said P450 gene is of rodent origin.

12. A method according to Claim 10 characterised in that said human P450 gene is selected from : CYP1A2; CYP2E1; or CYP3A4.

13. A method according to Claim 11 characterised in that said P450 gene is selected from: rodent CYP1A2; rodent CYP2E-1; or rodent CYP3A4.

14. A method according to Claims 1-14 characterised in that said tumour cell is selected from at least one of the following cancers: breast; pancreatic; ovarian; cervical; lung; hepatic; renal; testicular; prostate gastrointestinal; glioma; melanoma; bladder; lymphoma; leukaemia; epithelial, mesothelial; retinal.

15 A vector for use in the method according to Claims 1 – 14.

16. Acetaminophen in combination with a vector as defined in any of Claims 1 – 15 for use in the treatment of cancer.

17. A method for use in the treatment of cancer comprising:

i) administering to a mammal an effective amount of at least one vector, capable of transfecting at least one tumour cell, characterised in that said vector includes at least one P450 gene, or the effective part thereof, the expression of which is controlled by a promoter sequence, or the effective part thereof, which shows substantially tumour cell specific expression;

ii) administering an effective amount of at least one agent capable of modulating the amount of glutathione in said mammal; and

iii) administering a therapeutically effective amount of acetaminophen, or a structurally related derivative thereof.

18. A method according to Claim 17 characterised in that said agent is selected from at least one of: methionine; acetylcysteine.

19. A vector as defined in any of Claims 1 – 15 and a therapeutically effective amount of acetaminophen, or a structurally related derivative thereof, as a combined medicament for the simultaneous, separate or sequential use in the treatment of cancer.

20. A kit for use in the treatment of cancer comprising a vector as defined in any of Claims 1 - 15; acetaminophen; and, optionally an excipient, carrier or dilulent.

AMENDED SHEET

Claims

1 A method for use in the treatment of cancer comprising:

i) administering to a mammal an effective amount of at least one vector capable of transfecting at least one tumour cell characterised in that said vector includes at least one P450 gene, or the effective part thereof, the expression of which is controlled by a promoter sequence, or the effective part thereof, which shows substantially tumour cell specific expression; and

ii) administering a therapeutically effective amount of at least acetaminophen, or a structurally related derivative thereof.

2. A method according to Claim 1 characterised in that said mammal is human.

3. A method according to Claims 1 or 2 characterised in that said vector is a eukaryotic expression vector.

4. A method according to Claims 1 – 3 characterised in that said vector is a viral based vector.

5. A method according to Claim 4 characterised in that said vector is a hybrid viral vector.

6. A method according to Claim 4 or 5 characterised in that said viral based vector is selected from at least one of the following: adenovirus; retrovirus;adeno associated virus; herpesvirus; lentivirus; or baculovirus.

7. A method according to Claim 1 – 6 characterised in that said tumour promoter is selected from at least one of : TRP-1; HER2; HER3; ERBB2; ERBB3;

CEA; MUC1; or α-fetoprotein; Rous sarcoma virus long terminal repeat; cytomegalovirus promoter; murine leukaemia long terminal repeat; simian virus 40 early and late promoters; herpes simplex virus thymidine kinase promoter; prostate specific antigen promoter (PSA); zilin gene promoter; pancreatic amylase promoter; tyrosinase related peptide promoter; tumour rejection antigen precursor promoters.

8. A method according to Claim 7 characterised in that said promoter is a hybrid promoter of at least the effective parts of at least two tumour cell specific promoters.

9. A method according to Claims 1 – 8 characterised in that said P450 gene is of mammalian origin.

10. A method according to Claim 9 characterised in that said P450 gene is of human origin.

11. A method according to Claim 9 characterised in that said P450 gene is of rodent origin.

12. A method according to Claim 10 characterised in that said human P450 gene is selected from : CYP1A2; CYP2E1; or CYP3A4.

13. A method according to Claim 11 characterised in that said P450 gene is selected from: rodent CYP1A2; rodent CYP2E-1; or rodent CYP3A4.

14. A vector for use in the method according to Claims 1 – 13.

15. A method according to Claims 1 -14 characterised in that said tumour cell is selected from at least one of the following cancers: breast; pancreatic; ovarian; cervical; lung; hepatic; renal; testicular; prostate gastrointestinal; glioma; melanoma; bladder; lymphoma; leukaemia; epithelial, mesothelial; retinal.

16. The use of acetaminophen in the manufacture of a medicament for the treatment of cancer.

17. Acetaminophen in combination with a vector as defined in any of Claims 1 – 14 for use in the treatment of cancer.

18. A method for use in the treatment of cancer comprising:

i) administering to a mammal an effective amount of at least one vector, capable of transfecting at least one tumour cell, characterised in that said vector includes at least one P450 gene, or the effective part thereof, the expression of which is controlled by a promoter sequence, or the effective part thereof, which shows substantially tumour cell specific expression;

ii) administering an effective amount of at least one agent capable of modulating the amount of glutathione in said mammal; and

iii) administering a therapeutically effective amount of acetaminophen, or a structurally related derivative thereof.

19. A method according to Claim 18 characterised in that said agent is selected from at least one of: methionine; acetylcysteine.

20. A vector as defined in by any of Claims 1 – 14 and a therapeutically effective amount of at least acetaminophen, or a structurally related derivative thereof, as a combined medicament for the simultaneous, separate or sequential use in the treatment of cancer.

21. A kit for use in the treatment of cancer comprising a vector as defined in any of Claims 1 - 15; acetaminophen; and, optionally an excipient, carrier or dilulent.

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(57) Abstract

The invention hereindescribed relates to a form of cancer therapy which exploits the cytotoxic properties of acetaminophen when converted to NABQI by the metabolic activity of tumour cell specific P450; vectors for use in the delivery of P450 to tumour cells; and therapeutic compositions comprising said vectors.

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GENE THERAPY –1

1.Field of the Invention

This invention relates to a form of gene therapy known as genetically directed enzyme prodrug therapy (GDEPT).

2. Background of the Invention.

Current therapies to treat cancer involve, amongst other things, radiation therapy and chemotherapy each of which, although effective at retarding the growth of cancer cells, have significant disadvantages since each treatment is typically selective for cells that are actively dividing. Consequently, normal dividing cells are also destroyed resulting in significant undesirable side effects, such as nausea and immunosupression, the latter of which can lead to complications of secondary infections. In recent years research has focussed on providing selective treatments which lessen these undesirable side effects. One such therapy is GDEPT [1,2].

GDEPT is of particular interest with respect to the treatment of cancer in that it offers advantages over conventional chemotherapeutic methods of cancer treatment. In such conventional methods the drugs administered to the patient attack not only the targeted cancer cells but also normal cells. Destruction of cancer cells is achieved at the expense of inflicting damage on normal cells, creating serious side-effects. In treatment of cancer by GDEPT the objective is to create an anti-cancer drug in situ within the cancer cell while creating little or none in normal cells, thereby attacking the cancer cells while leaving the normal cells substantially unaffected. This is typically achieved by administration to the patient of a vector containing a gene for an enzyme which can convert a relatively non-toxic substance (commonly referred to as a prodrug) into a cytotoxic agent. The vector also contains a promoter, ie a DNA sequence constituting a switch for the gene, this promoter being responsive to a regulatory protein found solely in the cancer cells or to a greater extent in the cancer

cells than in normal cells. The gene is thus expressed substantially in the cancer cells so it is only (or mainly) in the cancer cells that the enzyme is produced and that conversion of the prodrug to the cytotoxic agent takes place. Formation of the cytotoxic agent therefore takes place primarily in the cancer cells. In this way the cancer cells are selectively attacked, with relatively little damage to normal cells.

In one example of the use of GDEPT in cancer treatment, the prodrug is 5-fluoro-cytosine (5-FC). 5-FC is itself relatively non-toxic to human cells but can be converted into a potent anti-cancer drug, 5-fluorouracil (5-FU), by the enzyme cytosine deaminase. A bacterial gene which expresses cytosine deaminase is incorporated in a viral vector in association with a promoter which is responsive to a regulatory protein that is characteristic of the particular type of cancer cell under attack. For instance, in treating breast cancer the promoter could be one which is responsive to the regulatory protein ERBB2 or in treating liver cancer one which is responsive to α-fetoprotein.

In known GDEPT techniques, difficulty has been encountered in achieving as high a degree of selectivity as is desirable, (ie in destroying cancer cells while limiting the damage to normal cells). This is at least partly due to the fact that normal cells may come under attack from cytotoxic agents which have been formed in the cancer cells but have found their way out of those cells, for example when the cells break down under the cytotoxic action of the drug.

In addition, some chemotherapeutic agents are selective for particular cell- cycle phases (eg G1, S, G2 or mitosis). It is desirable to provide chemotherapeutic agents that are not so restricted in their effects and can kill cells irrespective of the cell-cycle stage.

3. Detailed Background of the Invention

3.1. Acetaminophen as a Prodrug

Acetaminophen is a widely used mild pain reliever and antipyretic. However, it is a potentially dangerous drug in that an overdose of it can cause serious, even fatal, damage to the liver[3]. This is due to the fact that liver cells express a gene for a P450 enzyme, specifically CYP1A2, also to a much lesser extent CYP 2E1 and CYP 3A4. This enzyme can convert acetaminophen into a metabolite, N-acetylbenzoquinoneimine (NABQI), which is highly cytotoxic. For standard dosages of acetaminophen, the toxicity of NABQI is countered in the liver by conversion of NABQI into a non-toxic substance by reaction with glutathione, a normal component of human cells[4,5]. The supply of glutathione is however insufficient to deal with the large amounts of NABQI formed in liver cells after an overdose of acetaminophen and the cells are therefore then damaged or destroyed.

When acetaminophen constitutes the prodrug in GDEPT, the vector administered contains a gene for a p450 enzyme[7,8], preferably CYP1A2, and the cytotoxic agent formed in the cancer cells is NABQI. In contrast to other cytotoxic agents, NABQI causes little or no systemic toxicity.

3.2 Tumour Specific Gene Expression

It might have been expected that the use of acetaminophen as a prodrug in GDEPT would be impractical. Selective expression of the gene for the enzyme CYP1A2 in cancer cells could be effected by administration of a vector containing that gene in association with a promoter which is responsive to a regulatory protein found only in the cancer cells. The enzyme CYP1A2, created as a result of the entry into cells of that vector, would then convert acetaminophen into NABQI in the cancer cells and damage or destroy them. As in conventional GDEPT using prodrugs other than acetaminophen, selectivity between cancer cells and normal cells would be achieved

because entry of the vector into normal cells would not cause expression of the CYP1A2 gene contained in the vector since the normal cells do not contain the regulatory protein which activates the chosen promoter for the gene. In general, the normal cells would therefore not contain the enzyme CYP1A2 and would be unaffected by the presence of acetaminophen because in such cells it would not undergo intracellular conversion to NABQI. However, as mentioned above, normal liver cells naturally express a gene for CYP1A2. It would therefore be expected that administering a dose of acetaminophen high enough to create a level of NABQI in cancer cells capable of killing such cells could also result in the creation of sufficient NABQI in the normal liver cells to kill them too. Surprisingly, this is not so, probably due to a difference in the glutathione content of normal liver cells and that of cancer cells. It appears that most cancer cells may contain only about one-fifth of the glutathione present in normal liver cells. The concentration of the cytotoxic NABQI is therefore kept much lower in the normal liver cells than in the cancer cells because more NABQI can be detoxified, by combination of NABQI with glutathione, in the normal liver cells than in the cancer cells.

3.4 Gene Therapy Vectors & P450 Genes

The vector used in the present invention is one containing a gene for a p450 enzyme, preferably for CYP1A2, and a promoter which acts as a switch for that gene and which is responsive to a regulator protein characteristic of the type of cancer being addressed. The gene can be derived from human DNA (Ikeyak et al Molecular Endocrinology (1989), 3: 1399- 1408). However, it may be advantageous to use a P450 gene derived from non-human DNA, for example mouse DNA or hamster DNA. The P450 enzyme generated by the mouse gene is relatively unaffected by certain compounds, for example furaphylline, which act as inhibitors of the form of the enzyme CYP1A2 generated by the human gene. Administration of such inhibitors makes it possible to raise the dosage of acetaminophen above the normally safe dosage; an inhibitor such as furaphylline can protect the normal liver cells by inhibiting the form of P450 generated by expression in those cells of the human gene,

while having little or no effect on the form of P450 generated by expression of the mouse gene in the cancer cells. The level of NABQI in the normal liver cells is therefore diminished by such inhibitors while the level of NABQI in the cancer cells is relatively unaffected by the inhibitors.

With regard to non-viral delivery, synthetic uptake of DNA into mammalian cells can be facilitated by condensing it with lipids, proteins or peptides. These include, by example and not by way of limitation, polymers, dendrimers and cationic lipid delivery means (eg liposomes).

Liposomes are lipid based vesicles which encapsulate a selected therapeutic agent which is then introduced into a patient. The liposome is manufactured either from pure phospholipid or a mixture of phospholipid and phosphoglyceride. Typically liposomes can be manufactured with diameters of less than 200nm, this enables them to be intravenously injected and able to pass through the pulmonary capillary bed. Furthermore the biochemical nature of liposomes confers permeability across blood vessel membranes to gain access to selected tissues. Liposomes do have a relatively short half-life. So called STEALTH^R liposomes have been developed which comprise liposomes coated in polyethylene glycol (PEG). The PEG treated liposomes have a significantly increased half–life when administered intravenously to a patient. In addition STEALTH^R liposomes show reduced uptake in the reticuloendothelial system and enhanced accumulation selected tissues. In addition, so called immuno-liposomes have been develop which combine lipid based vesicles with an antibody or antibodies, to increase the specificity of the delivery of the DNA vector to a selected cell/tissue.

The use of liposomes as delivery means is described in US 5580575 and US 5542935.

Bacteria such as salmonella could be a more novel delivery vehicle. The DNA can also be coated on to microprojectiles and fired into the nuclei or target cells by a gene gun.

3.4 DNA Transfection

Many methods have been developed over the last 30 years to facilitate the introduction of DNA into cells which have greatly assisted, *inter alia,* our understanding of the control of gene expression.

Conventional methods to introduce DNA into cells are well known in the art and typically involve the use of chemical reagents, cationic lipids or physical methods. Chemical methods which facilitate the uptake of DNA by cells include the use of DEAE –Dextran (Vaheri and Pagano Science 175: p434) . DEAE-dextran is a negatively charged cation which associates and introduces the DNA into cells but which can result in loss of cell viability. Calcium phosphate is also a commonly used chemical agent which when co-precipitated with DNA introduces the DNA into cells (Graham et al Virology (1973) 52: p456).

The use of cationic lipids (eg liposomes (Felgner (1987) Proc.Natl.Acad.Sci USA, 84:p7413) has become a common method since it does not have the degree of toxicity shown by the above described chemical methods. The cationic head of the lipid associates with the negatively charged nucleic acid backbone of the DNA to be introduced. The lipid/DNA complex associates with the cell membrane and fuses with the cell to introduce the associated DNA into the cell. Liposome mediated DNA transfer has several advantages over existing methods. For example, cells which are recalcitrant to traditional chemical methods are more easily transfected using liposome mediated transfer.

More recently still, physical methods to introduce DNA have become effective means to reproducibly transfect cells. Direct microinjection is one such method which can deliver DNA directly to the nucleus of a cell (Capecchi (1980) Cell, 22:p479). This

allows the analysis of single cell transfectants. So called "biolistic" methods physically shoot DNA into cells and/or organelles using a particle gun (Neumann (1982) EMBO J, 1: p841). Electroporation is arguably the most popular method to transfect DNA. The method involves the use of a high voltage electrical charge to momentarily permeabilise cell membranes making them permeable to macromolecular complexes. However physical methods to introduce DNA do result in considerable loss of cell viability due to intracellular damage. These methods therefore require extensive optimisation and also require expensive equipment.

What is apparent from the above is that transfection of cells, either transiently or stably is a routine procedure undertaken by the man skilled in the art and is extensively referenced in academic publications, laboratory manuals and reference books. We have used both transient and stably transfected cell-lines to analyse the use of acetaminophen in GDEPT.

4. Statement of Invention

It is an object of the invention to provide a cancer therapy which reduces undesirable side effects of conventional cancer treatments.

It is a further object of the invention to provide a gene therapy based cancer treatment which targets cancer cells.

According to a first aspect of the invention there is provided a cancer therapy comprising:

i) administering to a mammal an effective amount of at least one vector capable of transfecting at least one tumour cell characterised in that said vector includes at least one P450 gene, or an effective part thereof, the expression of which is controlled by a promoter sequence, or the effective part thereof, which shows substantially tumour cell specific expression; and

ii) administering a therapeutically effective amount of at least acetaminophen, or a structurally related derivative thereof.

In a preferred method of the invention said mammal is human.

In a further preferred method of the invention said vector is an expression vector conventionally adapted for eukaryotic expression.

Typically said adaptation includes, by example and not by way of limitation, the provision of transcription control sequences (promoter sequences) which mediate cell/tissue specific expression. These promoter sequences may be cell/tissue specific, inducible or constitutive.

Promoter is an art recognised term and, for the sake of clarity, includes the following features which are provided by example only, and not by way of limitation. Enhancer elements are *cis* acting nucleic acid sequences often found 5' to the transcription initiation site of a gene (enhancers can also be found 3' to a gene sequence or even located in intronic sequences). Enhancers function to increase the rate of transcription of the gene to which the enhancer is linked. Enhancer activity is responsive to *trans* acting transcription factors (polypeptides) which have been shown to bind specifically to enhancer elements. The binding/activity of transcription factors(please see Eukaryotic Transcription Factors, by David S Latchman, Academic Press Ltd, San Diego) is responsive to a number of environmental cues which include, by example and not by way of limitation, intermediary metabolites (eg glucose, lipids) or enviromental effectors (eg light, heat,).

Promoter elements also include so called TATA box and RNA polymerase initiation site (RIS) sequences which function to select a site of transcription initiation. These sequences also bind polypeptides which function, *inter alia*, to facilitate transcription initiation selection by RNA polymerase.

Adaptations also include the provision of selectable markers and autonomous replication sequences which both facilitate the maintenance of said vector in either the eukaryotic cell or prokaryotic cell.

In addition adaptations which facilitate the expression of vector encoded genes include the provision of transcription termination/polyadenylation sequences. This also includes the provision of internal ribosome entry sites (IRES) which function to maximise expression of vector encoded genes arranged in bicistronic or multi-cistronic expression cassettes.

These adaptations are well known in the art. There is a significant amount of published literature with respect to expression vector construction. Please see, Sambrook et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, NY and references therein; Marston, F (1987) DNA Cloning Techniques: A Practical Approach Vol III IRL Press, Oxford UK.

In yet a further preferred method of the invention said vector is a viral based vector. Ideally said viral vector is selected from the following: adenovirus; retrovirus; adeno associated virus; herpesvirus; lentivirus; baculovirus.

Viral based vectors according to the invention may also include hybrid viral vectors which include advatageous features of selected viruses which facilitate, for example and not by way of limitation, viral infectivity, replication or expression of genes carried by said hybrid vector.

In a still further preferred method of the invention said promoter sequence is preferably selected from at least one of the following: TRP-1; HER2; HER3; ERBB2; ERBB3; CEA; MUC-1; α-fetoprotein; Rous sarcoma virus long terminal repeat; cytomegalovirus promoter; murine leukaemia long terminal repeat; simian virus 40 early and late promoters; herpes simplex virus thymidine kinase promoter;

prostate specific antigen promoter (PSA); zilin gene promoter; pancreatic amylase promoter; tyrosinase related peptide promoter; tumour rejection antigen precursor promoters.

In yet a further preferred method of the invention said P450 gene is of mammalian origin; ideally human. More ideally still said P450 gene is human CYP1A2. Alternatively said P450 gene is either human CYP2E1 or CYP3A4.

In yet still a further preferred method of the invention said P450 is of non-human origin. Ideally said P450 gene is derived from a rodent. More ideally still said rodent P450 gene is selected from homologous rodent genes encoding CYP1A2; CYP2E1; or CYP3A4.

GDEPT which uses rodent homologues of P450 are advantageous since inhibitors of human CYP1A2, for example, furaphylline, can be used in conjunction with acetaminophen. As noted previously, the rodent homologue of CYP1A2, is resistant to this inhibitor than the human form of the enzyme. This would therefore enable the use of elevated levels of acetaminophen since toxic amounts of NABQI would not be generated in the liver.

The administration of the vector according to the invention to the mammal is by conventional techniques. Typically this includes, by example and not by way of limitation, intravenous, intramuscular or intraperitoneal injection; or direct injection into the tumour tissue.

In yet a still further preferred method of the invention said tumour cell is selected from at least one on the following cancers: breast; pancreatic; ovarian; cervical; lung; hepatic; retinal; renal; testicular; prostate; gastointestinal; glioma; melanoma; bladder; lymphoma; leukaemia; epithelial; mesothelial;

In yet still a further preferred method of the invention there is provided the use of acetaminophen in the manufacture of a medicament for the treatment of cancer.

According to a further aspect of the invention there is provided a cancer therapy comprising:

i) administering to a mammal an effective amount of at least one vector, capable of transfecting at least one tumour cell, characterised in that said vector includes at least one P450 gene, or an effective part thereof, the expression of which is controlled by a promoter sequence, or the effective part thereof, which shows substantially tumour cell specific expression;

ii) adminstering an effective amount of at least one agent capable of modulating the amount of glutathione in said mammal; and

iii) administering a therapeutically effective amount of acetaminophen, or a structurally related derivative thereof.

Agents capable of increasing glutathione in the liver are well known in the art and include, by example and not by way of limitation, methionine, acetylcysteine.

An embodiment of the invention will now be described, by example only, and with reference to the following Table and Figures;

Table 1 represents the bystander effect on viability produced by incubating H1A2 MZ cells (stably transfected with human CYP1A2) with various cell lines in the presence of acetaminophen. Tumour cells or V79 MZ cells (non transfected, parental cells) were co-cultured overnight with various mixtures of H1A2 MZ cells (as indicated in the Table), washed with PBS and incubated with 4 mM acetaminophen in PBS for 6 h at 37°C. the cells were then washed once with PBS and maintained for either 24 or 48 h in culture medium appropriate to each cell type, as detailed in the Methods section. At 0, 6, 24 or 48 h the cells were washed in PBS and viability of the mixed cell population determined by trypan blue exclusion. The data shown

are mean values ± SEM of 4 separate determinations. Statistical significance was determined at each time point by comparing viability measurements in the mixed cell populations with both the viability determined at 0 h and in the absence of H1A2 MZ cells in the mixture 2 x 2 contingency tables using the 2-tailed Chi-squared test with Yate's correction. Viability was determined in a total of 1500 cells at each time point. Levels of significance are indicated as **$p<0.001$; ***$p<0.0001$;

Figure 1 is a graphical representation of an acetaminophen titration showing the sensitivity of COS cells transiently transfected with a vector incorporating mouse CYP1A2 and non-transfected control COS cells. Cell viability is monitored by tritiated thymidine incorporation;

Figure 2 is a cell viability time course of COS cells transiently transfected with mouse CYP1A2 and non-transfected control COS cells over a 24hr period. Acetaminophen concentration is 10mM. Cell viability is monitored by tritiated thymidine incorporation;

Figure 3 represents the effect of acetaminophen on the viability of stably transfected V79 MZ and H1A2 MZ cells. V79 MZ cells (stipplied bars) and H1A2 MZ cells (filled bars) were allowed to adhere to the wells of tissue culture plates overnight, washed with PBS and then incubated with various concentrations of acetaminophen for 6 h at 37 °C. Viability was determined by the ability of cells to exclude trypan blue. Data is represented as the mean ± SEM of 4 separate determinations and analysed by comparing the viability in H1A2 MZ cells with V79 MZ cells at each concentration of acetaminophen using the 2-tailed unpaired Student's t-test. Levels of significance are indicated as *$p<0.01$; ***$p<0.0001$;

Figure 4 represents the bystander effect on viability produced by incubating stably transfected, acetaminophen-activating H1A2 MZ cells with non- transfected parental V79 MZ cells. V79 MZ cells were co-cultured overnight with various mixtures of H1A2 MZ cells, washed with PBS and then incubated with 4 mM acetaminophen for

up to 6 h at 37°C. The cells were then washed in PBS and the viability of the mixed cell population determined by trypan blue exclusion. The cultures comprised V79 MZ cells only (open squares), and V79 MZ cells mixed with 5% (solid squares), 10% (open triangles), 25% (open circles) and 50% (solid circles) H1A2 MZ cells. In addition, the viability of H1A2 MZ cells in the presence (crosses) and absence (solid triangles) of acetaminophen is shown. Data is represented as the mean ± SEM of 4 separate determinations. Statistical analysis of the data at the 6 h time point is presented in Table 1.

Figure 5 represents the sub-cloning procedure to create the expression vector used in the transient transfection experiments; and

Figure 6 is the DNA sequence of the vector pEFPlink 6.

5. Materials and Methods

5.1.1. Recombinant DNA Techniques

The CYP1A2 cDNA is cloned downstream of a 544 bp fragment of the proximal 5' flanking region of the human ERBB2 gene in pBluescript II SK+ and then the chimeric minigene (ERBB2 promoter-CYP1A2 cDNA) is subcloned into a variety of eukaryotic expression vectors including:-

(a) the plasmid pPolyA (based on the commercial vector pcDNA [InVitrogen] from which the CMV promoter has been excised). Transfection is performed by application of plasmid DNA in the presence of cationic liposome complexes, either commercially obtained reagents such as Lipofectin (Life Technologies) or novel experimental agents (Genzyme). In order to allow for selection of genetically transformed clones the ERBB2-CYP1A2 plasmid is cotransfected at a 9:1 molar ratio with pSV2neo which encodes resistance to geneticin.

(b) the double copy retrovirus N2A, which allows conditional expression of the insert outside the transcription unit driven by the retroviral long terminal repeat promoter. Amphotropic retroviral stock is produced by packaging of the virus in GP + env AM12 cells.

(c) the adeno-associated virus vector psub 201, which when cotransfected into adenovirus-expressing cells together with pAAV/Ad leads to production of recombinant AAV that allows conditional expression of the insert in target cells.

The target cells in this example are human breast and pancreatic cancer cell lines which either over-express ERBB2 due to transcriptional upregulation or express normal (undetectable) levels.

5.1.2 Vector Construction for Transient Cell Transfection

A summary of the sub-cloning procedure is set-out in Figure 5. Briefly, the following steps were undertaken. CYP1A2 was subcloned from pCR™Bac (Invitrogen) by digestion with restriction enzymes EcoR1 and BamH1. This fragment was sub-cloned into EcoR1/BamH1 digested pEFPlink.6 which is derived from pEFPlink2 (Marais *et al* (1995) EMBO J 14: 3136 – 3145). pEFPlink.6 is altered by the selective removal of restriction sites and the provision of a more versatile multiple cloning site. The sequence of pEFPlink6 is given in Figure 4.

The sub-cloned CYP1A2 gene was sequenced to confirm its identity to the published CYP1A2 sequence. The vector containing the CYP1A2 gene is called pEF+cyp+.

Non-coding 5' and 3' sequences were then removed to generate restriction sites which facilitate the sub-cloning of CYP1A2 into a eukaryotic expresssion vector. CYP1A2 was PCR amplified and cut with Cla1 and Sma 1 and sub- cloned back into pEF+cyp+ to replace the original CYP1A2 gene. This removes much of the 3' untranslated region of the . This vector is called pEF+cyp. The 5' leader sequence

was deleted in the following way. Sequence specific primers were used to to amplify CYP1A2 from pEF+Plink+ and create a Nco1 site. It is preferable to use the ATG initiation codon in the Nco1 site to ensure efficient translation initiation of CYP1A2. The amplified fragment was cut with Nco 1 and HindIII and subcloned into pEFPlink6. This vector was sequenced to confirm the sub-cloning. pEFPlink was cut with Nco1 and HindIII and subcloned into pEF+cyp. This vector was called pEFcyp1A2.

This modified CYPA12 cDNA was then subcloned into the eukaryotic expression vector pMCEF which is derived from pEFPlink2 and contains the NeoR gene allowing selection in G418. The expression of the modified CYP1A2 cDNA is under the control of the elongation factor 1α promoter , (Marais *et al* Cancer Research (1996) 56: 4735 – 42). This was done by digestion of pEFcypA2 with Nco1 and Spe 1 and sub-cloning the fragment containing the modified CYP1A2 into pMCEF to generate the expression vector pMCEFcyp1A2.

pMCEFcyp1A2 incorporating mouse CYP1A2 was used in transient transfection experiments described below.

5.2 Transient Cell Transfection Using LipofectAMINE

LipofectAMINE (LPA)(Gibco BRL UK) is a lipid reagent that transfers DNA into cells and has proven to be very successful for transiently transfecting cells. We have found that we can achieve transfection frequencies approaching 50% (depending on cell-line and DNA construct). This compares very favourably with DEAE – dextran transfection which can only achieve approximately 0.5-2.5% efficiency. We estimate that LPA only results in 5% cell death when compared to 50% cell death when using DEAE- dextran.

COS cells are plated at 1.5 x 10^5 per well in 6 well tissue culture dishes the evening before transfection. Cells are left to grow overnight in Dulbecco-Vogt's Modified

Eagles Medium (DMEM) supplemented with 10% foetal calf serum, 2mM L-glutamine, penicillin(100U/ml) and streptomycin (100µg/ml). All tissue culture reagents can be obtained from Gibco BRL, Paisley, UK.

LPA/DNA complexes are prepared in accordance with the manufactures instructions. Briefly the following steps are undertaken. On the day of the transfection experiment dilutions of vector DNA are prepared in PBSA (0.4g KCl , 8.0g NaCl, 0.2g KH_2PO_4, 1.15g Na_2HPO_4 per litre) containing 0.5% (w/v) albumin . It is important not to use polypropylene reaction tubes as the LPA/DNA complex will adhere to the plastic.

Vector DNA is prepared at 0.4- 0.6 µg per transfection in 16µl of PBSA. Typically a stock of vector DNA is prepared at 0.025µg/ml in PBSA for use in transfection experiments. In experiments were more than one vector is used the same concentration of DNA is to be used and the volume adjusted accordingly. Note that when using multiple vectors it is important to mix the vectors prior to addition of LPA.

Typically, when preparing the LPA/DNA complex squares approximatelly 1cm x 1cm are marked onto the bottom of a petri dish corresponding to the number of transfection to be conducted. 10 – 12 µl PBSA is placed into the centre of each marked square to which 4-6 µl of LPA is added to give a total volume of 14-16µl. To this reaction mix is added 16µl stock vector DNA and the LPA/DNA complex is thoroughly mixed by passage through a micropipette tip by sucking the reaction mixture up and down 6 – 8 times. The reaction mixture is then left in the covered petri dish for approximtely 15 minutes.

During this incubation period, cells to be transfected are washed with serum free medium and then 800µl of serum free medium added prior to adddition of the complexed LPA/DNA.

To the LPA/DNA complex, 200µl of serum free medium is added to each LPA/DNA sample which is then gently added to the cells over a period of 3 – 4 seconds. Cells are returned to a 37^0C CO_2 incubator for 6 hours. Cells are washed twice in medium and 2.5ml of fresh medium is added to the cultures. Cultures can then be assayed at any time to monitor transgene expression.

The above described method provides reliable, high level expression of transgenes carried by vector DNA. The method is readily undertaken by the man skilled in the art.

5.3 Western Blotting

The level of expression of CYP1A2 is measured by Western blot analysis utilizing anti-peptide antibodies described in the literature (Edwards RJ et al, Biochem Pharmacol 1993; 46: 213-220 and Murray BP et al, Carcinogenesis 1993; 14: 585-592). These antibodies bind specifically to CYP1A2 in human liver microsomal fraction. In addition, one of the antibodies has been shown to bind readily to CYP1A2 expressed in a human B lymphoblastoid cell line transfected with a plasmid vector expressing human CYP1A2. In these cells the level of expression of human CYP1A2 was similar to that found in human liver, ie 8 pmols per mg protein (Edwards RJ et al, Carcinogenesis 1994; 15: 829-836). Microsomal fractions are prepared from tumour cells transfected with human CYP1A2, or cells transfected with an unrelated gene, eg cytosine deaminase, in the same vectors. Washed cells are disrupted using a Dounce homogeniser and the microsomal fraction prepared by ultracentrifugation and stored frozen at -80°C as described previously. Western blotting of microsomal fractions is performed as described previously (Boobis AR et al, Br J Clin Pharmacol 1980; 9: 11-19) employing enhanced chemiluminescence to maximise sensitivity.

5.4 Biochemical Activity of CYP1A2

In addition, the functional activity of expressed human CYP1A2 is determined in the tumour cells by measuring the rate of *O*-deethylation of phenacetin. At an appropriate substrate concentration (4 μM) this reaction is specifically catalysed by CYP1A2 in human liver microsomal fraction. Tumour cell microsomal fractions prepared as described above are incubated at 37°C in the presence of NADPH and the production of acetaminophen is determined by gas chromotography/negative ion chemical ionisation mass spectrometry using deuterated acetaminophen as internal standard. This highly sensitive assay easily measures CYP1A2 activity in small quantities (<10 μg) of human liver microsomal fraction which typically has an activity of 70 pmols/min/mg protein.

5.5 Cell Viability

Two methods were employed to monitor cell viability; exclusion of trypan blue by viable cells followed by cell counting; and incorporation of tritiated thymidine as a measure of DNA synthesis.

5.5.1 Trypan Blue Exclusion

For cell viability experiments 200,000 cells per well were plated on 12 well plates (Beckton-Dickenson, Oxford, UK), using the medium and conditions required by the tumour cell line and allowed to adhere overnight. After this the cells were washed with PBS before the addition of acetaminophen in 0.1 ml PBS (concentrations of 0.1 - 20 mM acetaminophen were dissolved in PBS by sonication) and then maintained at 37°C. At the appropriate time points, cells were removed from the plates by trypsinisation and collected by centrifugation. Cell viability was measured as ability to exclude trypan blue. Cells were counted at x100 magnification using an Improved Neubauer haemocytometer; all cell counts are the mean of duplicate determinations of five fields from duplicate experiments.

5.5 .2 ^{3}H-Thymidine Incorporation as a Measure of Cell Division

Transfected COS cells are either exposed to various concentrations of acetaminophen or incubated in the presence of 10mM acetaminophen and cell viability monitored with time. ^{3}H – thymidine (Amersham International UK, 1000Ci/mmol, 5μCi per assay) , is added to treated and control cultures and aliquots removed and acid precipitable counts assessed by liquid scintillation using a Beckton Liquid Scintillation Counter.

5.6 Glutathione depletion.

The intracellular glutathione content, comprising reduced and oxidised forms of glutathione is measured using a kinetic assay in which glutathione in the presence of glutathione reductase catalyses the continuous reduction of 5,5'-dithio-bis(2-nitrobenzoic acid) by NADPH. The rate of the reaction is proportional to the concentration of glutathione. The reaction is monitored at 412 nm and quantified by comparison with standards.

5.7 Measurement of [^{14}C] Acetaminophen

Covalent binding of radioactivity to cell protein following exposure to radioactive acetaminophen. Cells are incubated for up to 90 min with [^{14}C]acetaminophen. After washing, cellular protein is precipitated with trichloroacetic acid and the precipitate washed extensively with 80% methanol to remove unbound radioactivity. The protein pellet is digested in sodium hydroxide, neutralised, and the bound radioactivity measured by scintillation spectroscopy.

5.9 Cell Culture of Stably Transfected Cell-lines

V79 MZ Chinese hamster cells were maintained in Dulbecco-Vogt's modified Eagle's Medium (DMEM) supplemented with 10% foetal calf serum 9FCS), 2 mM L-glutamine, penicillin (100 U/ml and streptomycin (100 μg/ml) (all tissue culture reagents were obtained from Gibco BRL, Paisley, UK). H1A2 MZ cells, which are V79 MZ cells transfected with the human *CYP1A2* gene,[9] were also maintained in supplemented DMEM with the addition of geneticin at a concentration of 4 mg/ml. SK-OV-3 cells were grown in DMEM supplemented with 15% FCS and 2 mM L-glutamine, without the addition of antibiotics. RPMI-1640 medium with 10% FCS, 2 mM L-glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml) was required by the HCT116 cells. These cell lines were maintained at 37 °C with 100% humidity and 5% CO_2. MDA-MB-361 cells were grown in Leibovitz (L-15) medium supplemented with 15% FCS and 2 mM L-glutamine maintained at 37°C with 100% humidity and did not require CO_2. At confluence cells were removed from tissue culture flasks by inubation with trypsin-EDTA for 5 min, diluted 1 : 3-1 : 6 in fresh medium and seeded onto fresh flasks. The tumour cell lines were obtained from the European Collection of Cell Cultures.

5.9. Stably Transfected H1A2 MZ cells

The expression of human CYP1A2 in these cells was confirmed by measurement of 7-ethoxyresorufin O-deethylase and 7-methoxyresorufin O-deethylase activities on a cytosol-free protein fraction as described previously.[9] The respective values obtained of 5.4 ± 0.1 and 12.1 ± 0.2 pmol/min/mg protein (n=6) are similar to those reported previously, i.e. 6.5 and 12.8 pmol/min/mg protein[9] and no activity was detected in the parental V79 MZ cells. Further, the expression of human CYP1A2 in the cytosol-free protein fraction of H1A2 MZ cells, but not V79 MZ cells was also demonstrated by western blotting using an antibody specific for this P450 enzyme[10] (data not shown).

6. RESULTS

We show that transient transfection of COS cells using LPA with a vector carrying CYP1A2 under the control of a promoter which shows enhanced expression in tumour cells is capable of sensitising cells to therapeutically relevant concentrations of acetaminophen.

Figure 1 shows an acetaminophen titration comparing transfected COS cells with non-transfected control COS cells. Cells were transfected and allowed to recover and express CYP1A2 for 48hrs. Although non-transfected parental cell -lines show some sensitivity to acetaminphen, as measured by tritiated thymidine incorporation, transfected cells show a significant increase in sensitivity. The apparent sensitivity of control cells to acetaminophen can be attributed to the fact that COS cells contain a significant amount of p450 activity which will result in the production of NABQI in the presence of acetaminophen. However by increasing the basal levels of p450, by transfection with CYP1A2 it is shown that the administration of less acetaminophen results in reduced cell viability for an equivalent amount of acetaminophen, please see Figure 1, 4mM acetaminophen concentration.

Figure 2 shows a COS cell viability time course in response to 10mM acetaminophen. COS cells were transfected and compared to non- transfected control cells over a period of 24hrs. The extent of acetaminophen sensitivity increases markedly during the first 4 – 8 hrs. This correlates with the expression of CYP1A2 as monitored by western blot, results not shown. The sensitivity of transiently transfected COS cells is likely to be an underestimate of what may be achieved in stable cell-lines expressing CYP1A2 or *in vivo* in transgenic animal models since the vector DNA is susceptible to nuclease digestion resulting in a gradual decrease in potential sensitivity. This is, of course, is not shown by the control cells since the reduction in cell viability is the result of endogenous expression of p450 which is ongoing.

These results indicate that transiently transfected COS cells can be sensitised to acetaminophen by expression of a vector carrying a gene encoding CYP1A2. These results are confirmed by producing cell lines stably transfected with CYP1A2 which is described below.

Incubating H1A2 MZ cells, which stably express CYP1A2, with a range of concentrations of acetaminophen (0.1-20 mM) for 6 h allowed the determination of cytotoxic concentrations (Fig. 1). No cytotoxicity was observed in the absence of acetaminophen or with 0.1 mM acetaminophen. However, after exposure to 1 mM acetaminophen viability fell to 62%. Incubation with 4 mM acetaminophen resulted in a further reduction in viability to 8%. Higher concentrations of acetaminophen resulted in a similar amount of cell killing, Figure 3. In contrast, incubation of V79 MZ cells, which lack CYP1A2, with acetaminophen resulted in no loss of cell viability, Figure 3. Therefore, as 4 mM acetaminophen was the minimum dose to give the maximum effect, this concentration was selected for further experiments.

To determine if the toxic metabolite produced by CYP1A2 had a cytotoxic effect on bystander cells incapable of activating acetaminophen, H1A2 MZ cells were mixed with V79 MZ cells before exposure to 4 mM acetaminophen. The percentage decline in total cell viability greatly exceeded the percentage of acetaminophen-activating cells in the mixture indicating a significant bystander effect, Figure 4. In the presence of 5% H1A2 MZ cells the viability of the mixed cell population was reduced to 52%, and as the proportion of H1A2 MZ cells was increased the number of viable cells in the mixture declined (Fig. 4, Table 1) and a near maximal effect was found with a mixture of an equal number of V79 MZ and H1A2 MZ cells, Figure 4. In contrast, no decline in cell viability was observed in V79 MZ cells incubated in 4 mM acetaminophen or H1A2 MZ cells incubated in phosphate-buffer saline (PBS) without acetaminophen (Figure 4, Table 1).

The susceptibility of tumour-derived cells to cytotoxicity produced by the activation of acetaminophen was investigated by mixing H1A2 MZ cells with SK-OV-3,

HCT116 and MDA-MB-361 cells and incubating them in the presence of 4 mM acetaminophen for 6 h. It was found that like V79 MZ cells, SK-OV-3 cells were highly sensitive as viability fell progressively as the prorportion of H1A2 MZ cells was increased (Table 1). HCT116 cells showed a disproportionate decrease in viability when incubated with 5 and 10% H1A2 MZ cells, although with 50% H1A2 MZ cells the amount of cell killing could be accounted for by loss of H1A2 MZ cells alone (Table 1). However, MDA-MB-361 cells appeared to be highly resistant to cytotoxicity as the reduction in cell viability was similar to the proportion of H1A2 MZ cells present (Table 1).

To determine if the residual viable cells were programmed to die, as we have found previously,[11] attempts were made to culture the various cell types in normal growth medium after they had been exposed to acetaminophen for 6 h in the presence of the activating H1A2 MZ cells. It was found that after 24 h in culture, the viability of V79 MZ, SK-OV-3 and HCT116 cells mixed with as little as 5% H1A2 MZ cells fell to zero (Table 1). Only MDA-MB-361 cells showed resistance to cell killing, although even with these cells viability was reduced to 18% in cultures comprising an equal number of MDA-MB-361 and H1A2 MZ cells and no viable cells were found after 48 h (Table 1). Nevertheless, with fewer H1A2 MZ cells present viability increased with time, presumably as the number of dividing MDA-MB-361 cells increased during culture.

7. DISCUSSION

Acetaminophen can be activated by oxidation catalysed by human CYP1A2 to form the cytotoxic compound NABQI. The combination of acetaminophen as prodrug and CYP1A2 as activating enzyme has a potential application in GDEPT. It has been demonstrated here that a sufficient amount of NABQI produced in cells transfected with human CYP1A2 to cause cyotoxicity. Further, enough NABQI is released from activating cells to cause cytotoxicity in neighbouring cells. Such a bystander effect was readily demonstrated in V79 MZ cells. However, human tumour-derived cells showed a range of sensitivities. An extensive bystander effect was found using SK-

OV-3 cells, an ovarian tumour derived cell line, whereas, HCT-116 cells, which are derived from a colon tumour, appeared to be somewhat more resistant to the cytotoxic effect of NABQI when assessed immediately after a 6 h incubation period. However, both of these tumour cell types appeared to have been markedly damaged by exposure to NABQI as they failed to survive in culture. On the other hand, the MDA-MB-361 cells, which originate from a breast tumour, displayed resistance of the cytotoxic effect of NABQI, with only those cells exposed to the highest concentration being affected.

NABQI is capable of arylating and oxidising protein thiol groups, although studies with thiol reductants such as dithiothreitol[11] and N-acetylcysteine[12] suggested that the reversible oxidation of thiols (i.e. "oxidative stress") rather than arylation is responsible for cell death from acetaminophen.[6] Irrespective of the exact mechanism, the key factors that determine the toxicity of acetaminophen are the rate of production of NABQI as determined by the activity of the relevant cytochrome P450 enzymes and the starting level and extent of depletion of GSH. Thus rat liver is relatively resistant to acetaminophen, but not pre-formed NABQI, because the rate of formation of the reactive metabolite is insufficient, even at very high concentrations of acetaminophen, to deplete GSH.[13] In contrast, hamsters are very sensitive because acetaminophen is rapidly and extensively oxidised to NABQI. Human liver hepatocytes exhibit a range of sensitivities which correlate with the rate of oxidation of acetaminophen to NABQI.[13] Toxicity in overdosed individuals is confined to the liver which is the only organ that has the required level of enzyme activity to generate NABQI to deplete GSH, a prerequisite for cell damage and death. The hepatotoxicity of acetaminophen can be greatly increased in all species by prior depletion of GSH with chemicals such as diethylmaleate.[14] Thus the toxicity of acetaminophen is dependent upon the balance between the activity of the NABQI-generating enzyme and the concentration of GSH.

GSH concentrations in breast tumours (913nmol/g tissue) are two-fold greater than in normal breast tissue[15] but are less than 20% of those found in normal human liver

(>5000 nmol/g tissue). Thus if tumour cells can be made to express NABQI-producing activity similar to human liver, therapeutic doses of acetaminophen should be selectively cytotoxic to the tumour cells. In addition, it will be possible to selectively protect the liver from any toxic effects of acetaminophen by oral administration of GSH precursors such as methionine or N-acetylcysteine[16] that elevate GSH in the liver but not other tissues.[17]

It might be possible to increase the efficiency of the enzyme activating system by replacing human CYP1A2 with another P450 enzyme with a greater capacity for acetaminophen activation. Although human CYP2E1 and CYP3A4 are known to catalyse this reaction,[7] the rates relative to CYP1A2 at high concentrations of acetaminophen have yet to be determined. Alternatively, the orthologous rodent forms of CYP1A2, CYP2E1 or CYP3A4 may provide the source of a more efficient enzyme.

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Table 1.

Cell type	H1A2 MZ cells (%)	Viability (%) 0 h	6 h	24 h	48 h
V79 MZ	0	99 ± 1	94 ± 7	98 ± 1	-
	5	98 ± 1	52 ± 5***	0 ± 0***	-
	10	98 ± 1	39 ± 6***	0 ± 0***	-
	25	97 ± 2	20 ± 4***	0 ± 0***	-
	50	96 ± 3	15 ± 7***	0 ± 0***	-
SK-OV-3	0	97 ± 1	98 ± 2	96 ± 3	-
	5	98 ± 1	55 ± 2***	0 ± 0***	-
	10	98 ± 1	43 ± 7***	0 ± 0***	-
	25	97 ± 1	22 ± 11***	0 ± 0***	-
	50	98 ± 1	16 ± 9***	0 ± 0***	-
HCT116	0	96 ± 1	96 ± 5	96 ± 2	-
	5	98 ± 1	69 ± 9***	0 ± 0***	-
	10	98 ± 1	60 ± 6***	0 ± 0***	-
	25	97 ± 1	56 ± 4***	0 ± 0***	-
	50	98 ± 1	42 ± 6***	0 ± 0***	-
MDA-MB-361	0	96 ± 1	98 ± 4	95 ± 3	97 ± 1
	5	98 ± 2	85 ± 2**	84 ± 7**	92 ± 4
	10	96 ± 3	74 ± 7***	81 ± 9**	94 ± 7
	25	97 ± 1	70 ± 11***	78 ± 9***	91 ± 6
	50	98 ± 3	42 ± 4***	18 ± 5***	0 ± 0***

5

Claims

1 A method for use in the treatment of cancer comprising:

i) administering to a mammal an effective amount of at least one vector capable of transfecting at least one tumour cell characterised in that said vector includes at least one P450 gene, or the effective part thereof, the expression of which is controlled by a promoter sequence, or the effective part thereof, which shows substantially tumour cell specific expression; and

ii) administering a therapeutically effective amount of at least acetaminophen, or a structurally related derivative thereof.

2. A method according to Claim 1 characterised in that said mammal is human.

3. A method according to Claims 1 or 2 characterised in that said vector is a eukaryotic expression vector.

4. A method according to Claims 1 – 3 characterised in that said vector is a viral based vector.

5. A method according to Claim 4 characterised in that said vector is a hybrid viral vector.

6. A method according to Claim 4 or 5 characterised in that said viral based vector is selected from at least one of the following: adenovirus; retrovirus;adeno associated virus; herpesvirus; lentivirus; or baculovirus.

7. A method according to Claim 1 – 6 characterised in that said tumour promoter is selected from at least one of : TRP-1; HER2; HER3; ERBB2; ERBB3;

CEA; MUC1; or α-fetoprotein; Rous sarcoma virus long terminal repeat; cytomegalovirus promoter; murine leukaemia long terminal repeat; simian virus 40 early and late promoters; herpes simplex virus thymidine kinase promoter; prostate specific antigen promoter (PSA); zilin gene promoter; pancreatic amylase promoter; tyrosinase related peptide promoter; tumour rejection antigen precursor promoters.

8. A method according to Claim 7 characterised in that said promoter is a hybrid promoter of at least the effective parts of at least two tumour cell specific promoters.

9. A method according to Claims 1 – 8 characterised in that said P450 gene is of mammalian origin.

10. A method according to Claim 9 characterised in that said P450 gene is of human origin.

11. A method according to Claim 9 characterised in that said P450 gene is of rodent origin.

12. A method according to Claim 10 characterised in that said human P450 gene is selected from : CYP1A2; CYP2E1; or CYP3A4.

13. A method according to Claim 11 characterised in that said P450 gene is selected from: rodent CYP1A2; rodent CYP2E-1; or rodent CYP3A4.

14. A vector for use in the method according to Claims 1 –13.

15. A method according to Claims 1 -14 characterised in that said tumour cell is selected from at least one of the following cancers: breast; pancreatic; ovarian; cervical; lung; hepatic; renal; testicular; prostate gastrointestinal; glioma; melanoma; bladder; lymphoma; leukaemia; epithelial, mesothelial; retinal.

16. The use of acetaminophen in the manufacture of a medicament for the treatment of cancer.

17. Acetaminophen in combination with a vector as defined in any of Claims 1 – 14 for use in the treatment of cancer.

18. A method for use in the treatment of cancer comprising:

i) administering to a mammal an effective amount of at least one vector, capable of transfecting at least one tumour cell, characterised in that said vector includes at least one P450 gene, or the effective part thereof, the expression of which is controlled by a promoter sequence, or the effective part thereof, which shows substantially tumour cell specific expression;

ii) administering an effective amount of at least one agent capable of modulating the amount of glutathione in said mammal; and

iii) administering a therapeutically effective amount of acetaminophen, or a structurally related derivative thereof.

19. A method according to Claim 18 characterised in that said agent is selected from at least one of: methionine; acetylcysteine.

20. A vector as defined in by any of Claims 1 – 14 and a therapeutically effective amount of at least acetaminophen, or a structurally related derivative thereof, as a combined medicament for the simultaneous, separate or sequential use in the treatment of cancer.

21. A kit for use in the treatment of cancer comprising a vector as defined in any of Claims 1 - 15; acetaminophen; and, optionally an excipient, carrier or dilulent.

Figure 1

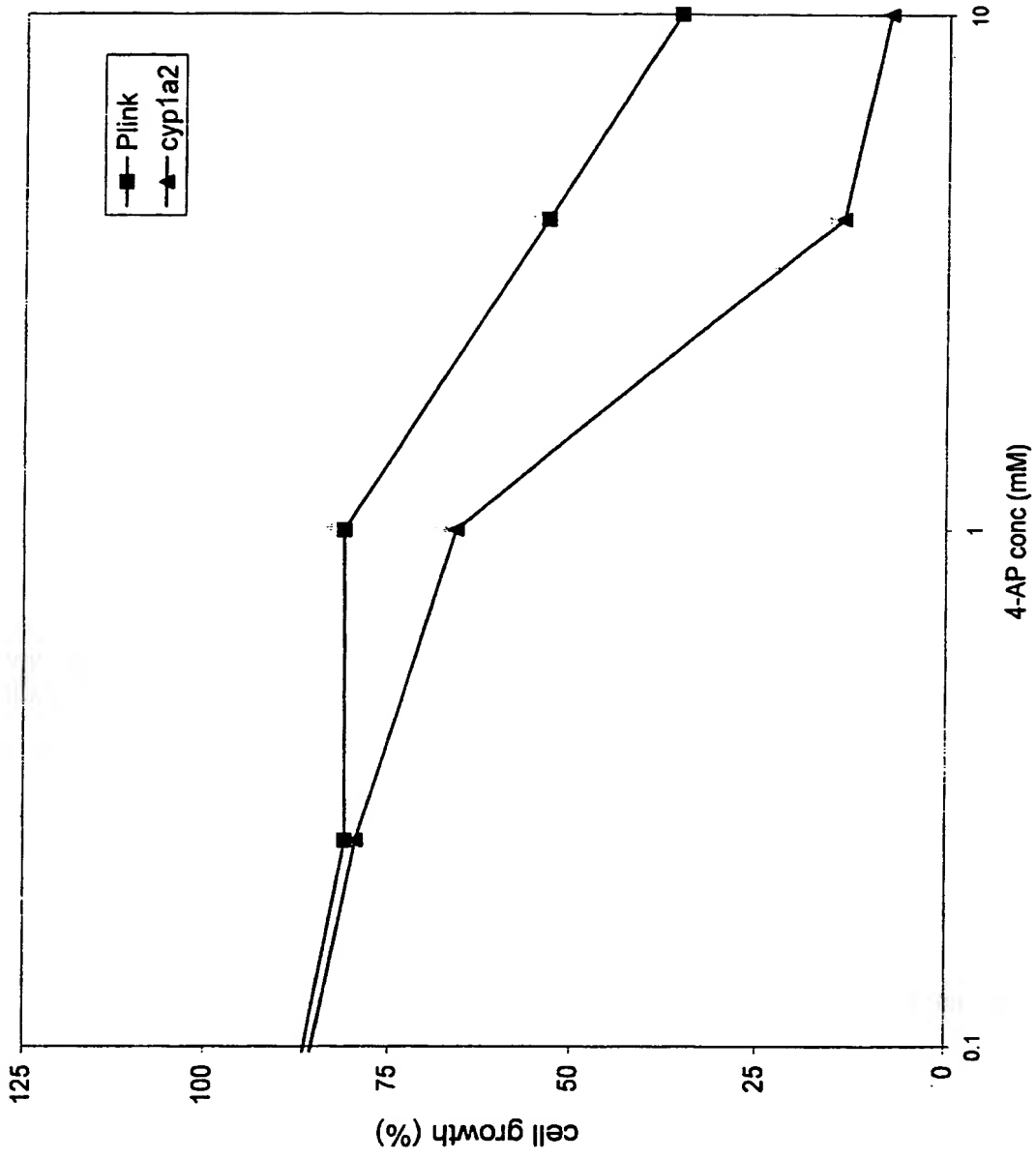

Figure 2

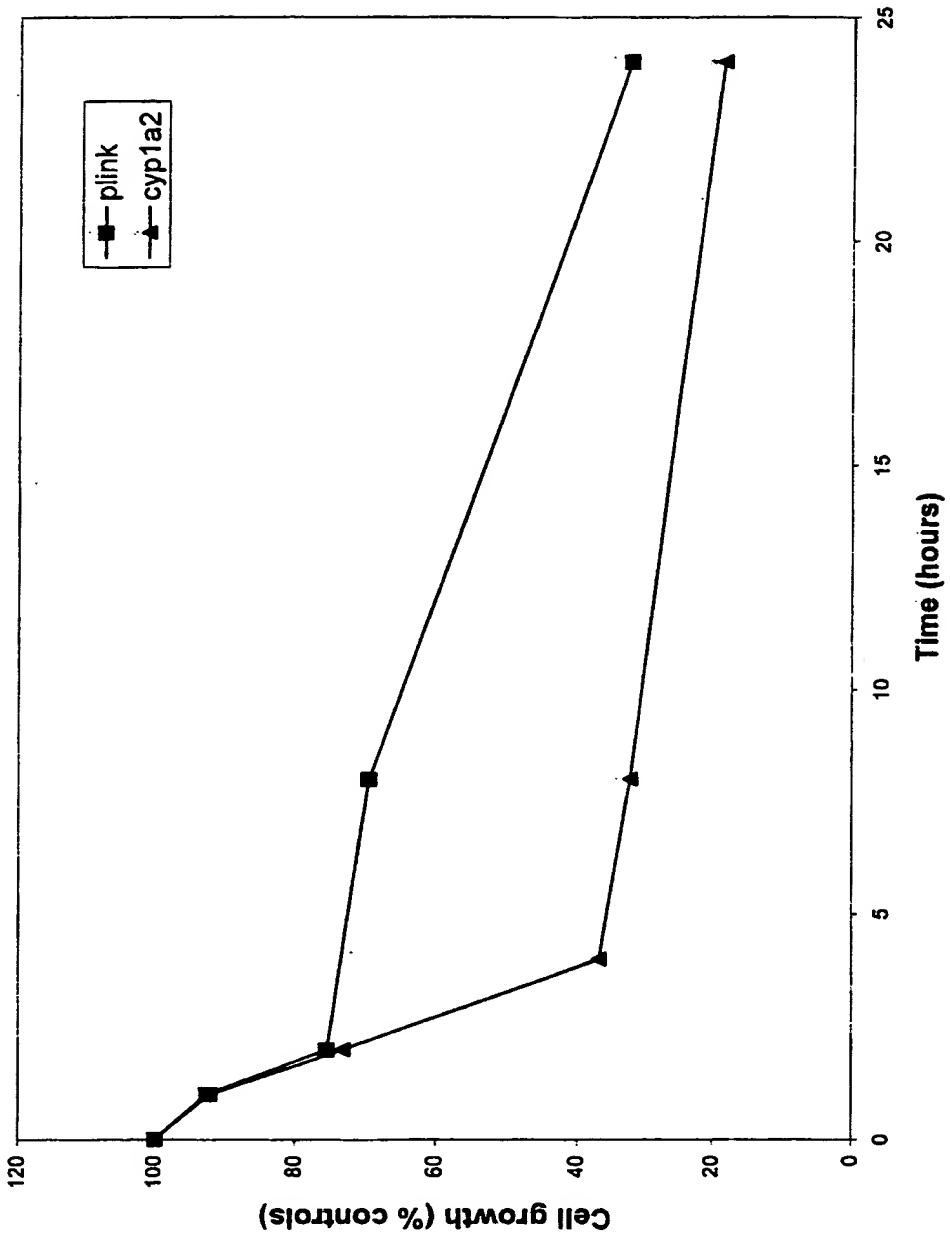
plink
cyp1a2
Cell growth (% controls)
120
100
80
60
40
20
0
Time (hours)
0
5
10
15
20
25

FIGURE 3

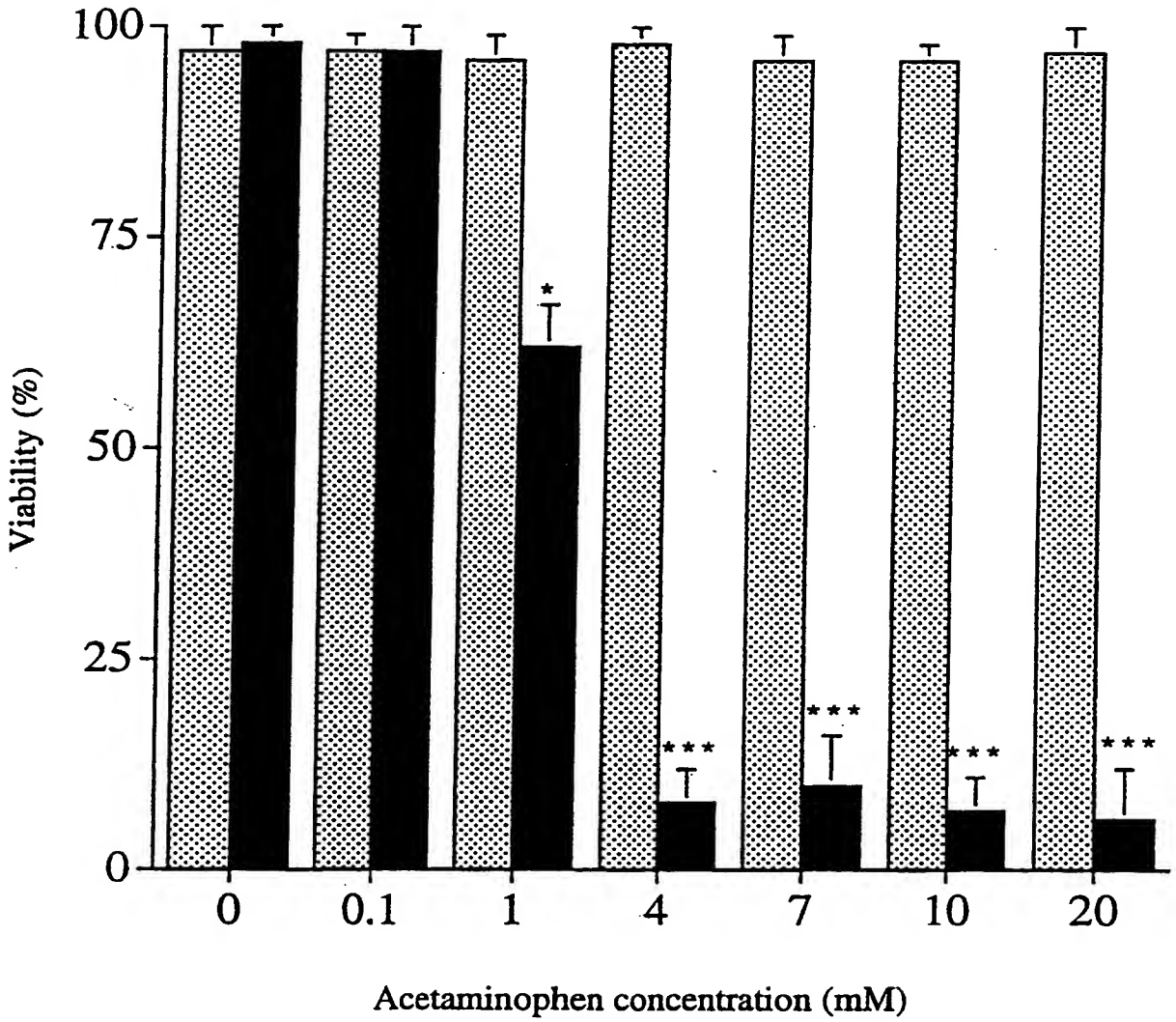
100
75
50
25
0
Viability (%)
*
***
***
***
***
0
0.1
1
4
7
10
20
Acetaminophen concentration (mM)

FIGURE 4

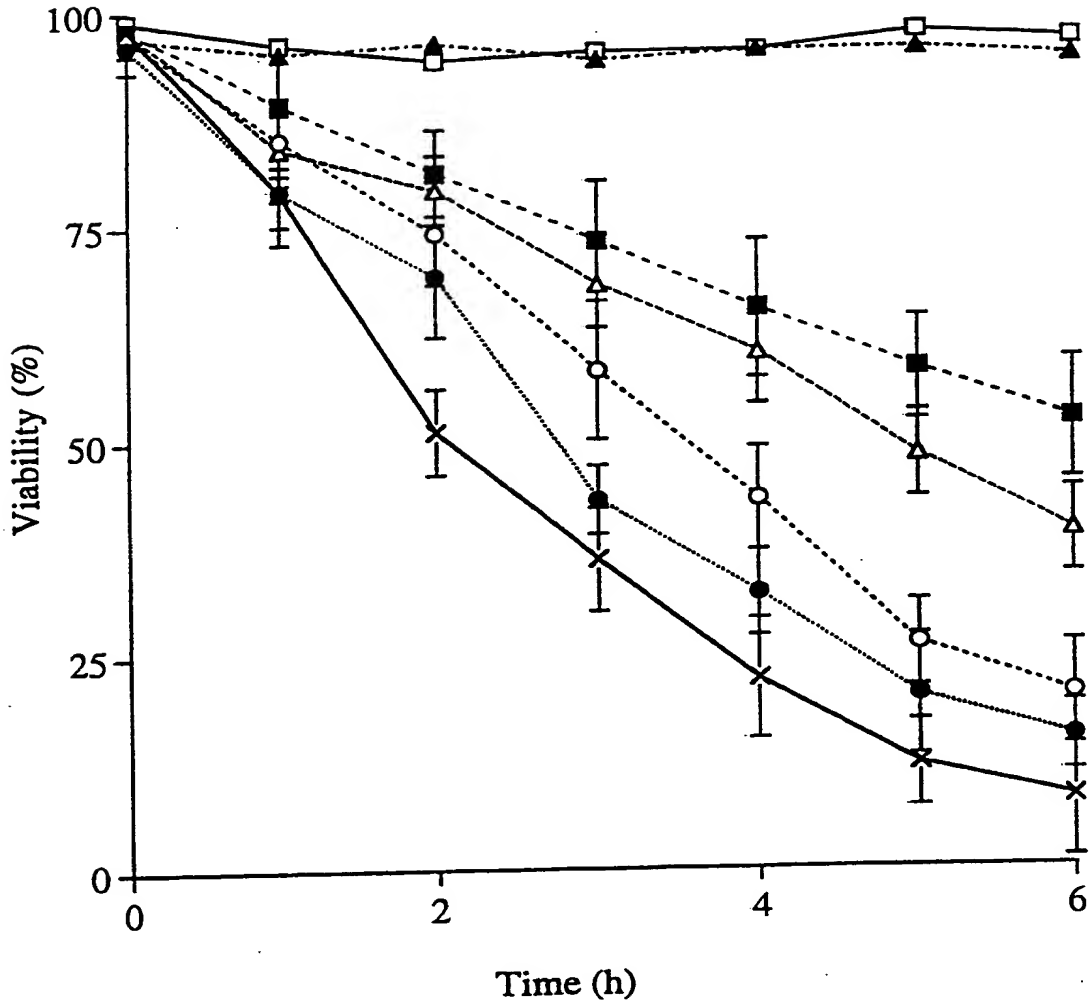

Figure 5

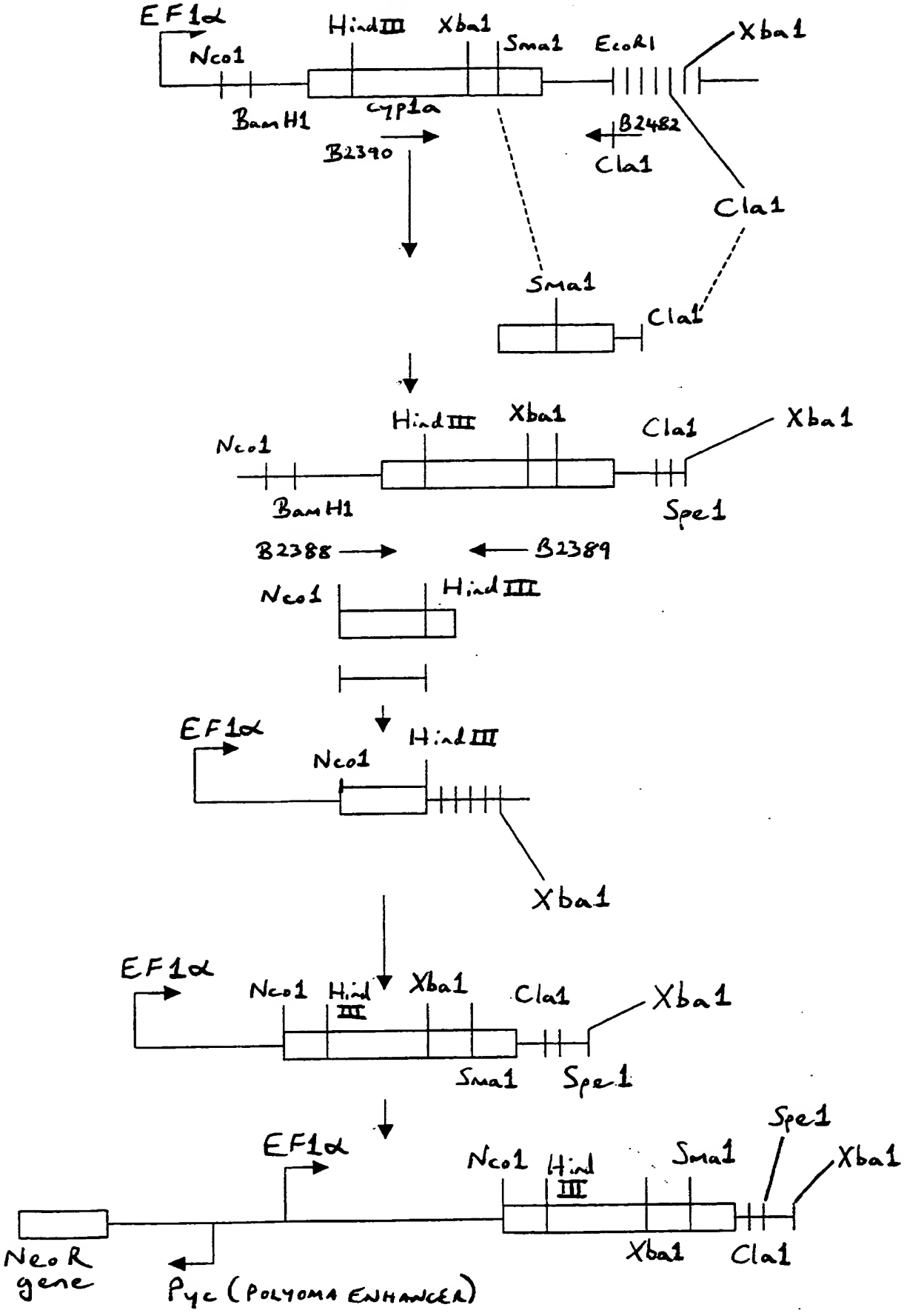
EF1α
Nco1
BamH1
Hind III
Xba1
Sma1
cyp1a
B2390
EcoR1
B2482
Cla1
Xba1
Cla1
Sma1
Cla1
Hind III
Xba1
Cla1
Xba1
Nco1
BamH1
Spe1
B2388
B2389
Nco1
Hind III
EF1α
Nco1
Hind III
Xba1
EF1α
Nco1
Hind III
Xba1
Cla1
Xba1
Sma1
Spe1
EF1α
Nco1
Hind III
Sma1
Spe1
Xba1
Xba1
Cla1
Neo R gene
Pyc (POLYOMA ENHANCER)

FIGURE 6

GTTTGACAGCTTATCATCGACTGCACGGTGCACCAATGCTTCTGGCGTCAGGCAGCCATCGGAAGCTGTGGTATGGCTGT
GCAGGTCGTAAATCACTGCATAATTCGTGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGACATCAT
AACGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCGGGCTCGTATAATGTGTGGAATTGTGAGCGGA
TAACAATTTCACACAGGAAACAGACCATGGAGCAGAAGCTGATTTCCGAGGAGGACCTGGGATCCGAATTCAAGCTTCCC
GGGGTCGACATCGATTAGACTAGTCTAGACGGCGGCGGCAGCTTGGCTGTTTTGGCGGATGAGAGAAGATTTTCAGCCTG
ATACAGATTAAATCAGAACGCAGAAGCGGTCTGATAAAACAGAATTTGCCTGGCGGCAGTAGCGCGGTGGTCCCACCTGA
CCCCATGCCGAACTCAGAAGTGAAACGCCGTAGCGCCGATGGTAGTGTGGGGTCTCCCCATGCGAGAGTAGGGAACTGCC
AGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCT
GAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCAT
AAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGTTTCTACAAACTCTTTTTGTTTAT
TTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAG
AGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGA
AACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTA
AGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTA
TCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGT
CACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGG
CCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGC
CTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTACAGCAATGGCAAC
AACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATA
AAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGG
TCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGC
AACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTT
ACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTC
ATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGA
TCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAG
AGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAG
TTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAG
TGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGG
GTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCC
ACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCC
AGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGT
CAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCAC
ATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAG
CCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGT
GCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATACACTCCGC
TATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTC
CCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACG

CGCGAGGCAGCAGATCAATTCGCGCGCGAAGGCGAAGCGGCATGCATTTACGTTGACACCATCGAATGGTGCAAAACCTT
TCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCG
CAGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAA
AAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCT
GATTGGCGTTGCCACCTCCAGTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAAC
TGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCG
CAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGT
TCCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGG
GCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTG
CGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCAT
GTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCAGATGG
CGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATACGACGATACC
GAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCG
CTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCC
TGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTG
GAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGGGCGAATTGATCTG